

THE APPLICATIONS OF MOLECULAR EVOLUTION TO SYSTEMATICS: RATES, REGULATION, AND THE ROLE OF NATURAL SELECTION¹

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The development of biochemical methods for comparing the amino acid sequences of homologous proteins from different species has provided a powerful tool for investigations of evolution and systematics. Perhaps the most intriguing (and most controversial) result of the comparative studies of proteins using these methods has been the discovery that sequences may change at nearly constant rates (Wilson, Carlson & White, 1977). This is not to imply that different genes or proteins evolve at the same rate: rather, each class of proteins has its own characteristic rate (Dickerson, 1971). (Serum albumin, for example, has evolved more rapidly than cytochrome *c*, but serum albumin has evolved at approximately the same rate among all species of mammals tested, as has cytochrome *c*.) The degree of rate constancy has been the subject of intense debate, but the most current evidence indicates that the variation in evolutionary rate for a given protein is only about twice the variation expected for a totally stochastic process such as radioactive decay (Fitch, 1976). Within these limits, then, a given macromolecular sequence may be used as an evolutionary "clock."

The empirical discovery that molecules can be evolutionary "clocks" has been applied to a variety of problems in evolution and systematics. Most frequently, sequence data for a given protein from a number of species has been used to reconstruct phylogenetic trees depicting the probable order of branching of the lineages leading to modern species from a common ancestor. For example, Boulter and his colleagues have reconstructed a possible phylogeny for the flowering plants based on the cytochrome *c*, plastocyanin, and ferredoxin sequences of representative species (Boulter, 1974). In addition, phylogenetic analysis of sequences of 5S and 16S ribosomal RNA from chloroplasts, bacteria, blue-green algae, and cytoplasm of green plants has confirmed that chloroplasts evolved from photosynthetic prokaryotes living as endosymbionts within the cytoplasm of primitive heterotrophic plants (Margulis, 1970; Bonen & Doolittle, 1976; Zablén et al., 1975; Hori, 1975).

Molecular phylogenies may also indicate evolutionary times of divergence if the divergence time for at least one branching event in a tree can be accurately estimated from paleontological or biogeographical evidence. The use of cytochrome *c* sequences to estimate times of divergence for flowering plants poses a fascinating, and still unresolved dilemma. If the cytochromes *c* of plants are evolving at the same rate as those of vertebrates, which have a unit evolutionary period of about 20 million years, then the intraordinal divergence times for flower-

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ing plants would be about 240 million years ago (Ramshaw et al., 1972; Boulter et al., 1972). Since the first clearly authentic fossils of flowering plants occur about 130 million years ago (Sporne, 1971), an alternative interpretation of the data is that morphologically plant cytochromes *c* have evolved twice as fast as those of vertebrates (Cronquist, 1976). Workers in this field are now giving serious attention to the possibility that the origin of flowering plants is more ancient than is indicated by the available fossil evidence (Wilson, Carlson & White, 1977). This situation may be analogous with the origin of mammals, in that the group is very ancient, while adaptive radiation within the group is more recent.

The molecular evolutionary approach may also have revealed an important mechanism for evolution at the organismal level. This discovery results from the observed discrepancy between the evolution of macromolecules and the evolution of organisms. The comparison of humans and chimpanzees at both the macromolecular level and organismal levels indicates that the two species differ to an extent considered familial in morphology, behavior, and adaptive strategy, while their protein sequences differ by less than one percent—a level of difference characteristic of sibling species of *Drosophila* or mammals (King & Wilson, 1975). Major adaptive changes may thus be based on molecular events other than sequence changes in structural genes. What sorts of events might these be? Experimental studies of bacterial evolution have demonstrated that major phenotypic changes—in the bacterial case the acquisition of a new metabolic activity—depend on an increase in the effective concentration of a protein which previously limited the rate of metabolism of a given substrate, rather than on a qualitative change in the substrate specificity of any protein (Lerner et al., 1964). These quantitative effects could be due to point mutations in regulatory genes or to chromosomal rearrangements such as duplications and translocations (Wilson, 1975). The observation that rates of karyotypic change are fastest in vertebrate groups with the most rapid phenotypic evolution may indicate that major adaptive shifts in the evolution of multicellular organisms are frequently associated with chromosomal rearrangements (Wilson et al., 1975).

The independence of the evolution of organisms and the evolution of their structural genes may provide a new perspective for investigating the evolutionary roles of natural selection versus random fixation of selectively neutral alleles. If the random fixation of neutral substitutions were principally responsible for sequence evolution of genes and proteins, it would follow that the rate of sequence evolution would depend primarily on the mutation rate, which is assumed to be constant with time. The “neutral” hypothesis is thus consistent with the observation that sequence evolution depends on calendar time. In addition, the “neutral” hypothesis accounts for the observation that proteins can differ greatly in sequence without differing appreciably in biological activity. At the same time, it is unequivocally established that natural selection acts at the level of the organism, and that this selective pressure varies greatly over time and space. It is tempting to suggest a hypothesis consistent with—though in no way proven by—each of these observations: that fixed substitutions in the variable regions of structural genes have generally not been subject to selective

pressures, since these substitutions are largely irrelevant to the adaptive success of the organism. Instead, natural selection at the level of the organism may be reflected at the molecular level in both rapid elimination of deleterious mutations in regulatory systems and fixation of occasional adaptive changes in loci or patterns of genome organization controlling the expression of structural genes.

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